Cross-regulation of *Arabidopsis* root growth by plant hormones auxins and ethylene

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Abstract

We investigated the cross-talk between auxin (IAA) and ethylene in the control of root growth of *Arabidopsis* plants (*Arabidopsis thaliana*). The root growth of ethylene insensitive *etr1-1* and auxin insensitive *tir1* mutants under the effect of IAA and 1-methylcyclopropene (1-MCP, inhibitor of ethylene perception) was compared, respectively, with that of the wild type ecotype Columbia (Col-0). Roots of Col-0 were shorter than those of the *etr1-1* mutant. The addition of IAA (5.7×10^{-6} M) to the growth medium led to 20 % root shortening in Col-0 plants, but not in *etr1-1* mutants. Thus, the capacity of plants for ethylene perception contributes to the control of root length and its sensitivity to IAA. Roots of *etr1-1* were less heavy than in Col-0, indicating that ethylene maintains root mass accumulation. Treatment with IAA caused a decrease in root mass of both genotypes (resulting in a 25 and 10 % decline in the root mass of Col-0 and *etr1-1* as compared to the corresponding control), suggesting that IAA may influence root biomass accumulation independently of ethylene. However, sensitivity to ethylene increases plant responsiveness to IAA. Mutation in the auxin receptor decreased the sensitivity of roots to inactivation of ethylene receptors: treatment with 1-MCP resulted in a 40 % decline in the root mass of Col-0 and only a 10 % decrease in *tir1.* The decrement in sensitivity to auxins in the *tir1* mutant decreased the responsiveness of root biomass to 1-MCP-treatment. These data suggest an additive action of ethylene and auxins on accumulation of root biomass.

Keywords: *Arabidopsis thaliana*, *etr1-1*, *tir1*, ethylene, IAA, root growth, 1-MCP.

Introduction

Regulation of root growth plays an important role in plant adaptation to the environment and particularly to heterogeneous distribution of ions, which is a general feature of natural soil. When the growing roots reach patches with an increased concentration of mineral nutrients, their elongation is inhibited, which contributes to effective uptake of ions (Trapeznikov et al., 2003; Ivanov, 2009). The importance of root growth reaction determines the interest in discovering mechanisms responsible for its regulation. Ethylene and auxins are well known to inhibit root elongation (Takahashi, 2013). The similarity of plant response to either of these two hormones indicated their cross-talk in implementation of inhibitory action on root growth (Muday et al., 2012). The results of comparative transcriptomic analysis highlighted that cross-talk between ethylene and auxin is due to the mutual regulation of these hormone pools (Stepanova et al., 2007). Auxins have been shown to be capable of stimulating ethylene production (Woeste et al., 1999), while ethylene, in turn, influences the accumulation and distribution of auxins through effects on

Citation: Korobova, A., Vasinskaya, A., Kirpichnikova, A., Shishova, M., and Kudoyarova, G. 2018. Cross-regulation of *Arabidopsis* root growth by plant hormones auxins and ethylene. Bio. Comm. 63(4): 256–260. https://doi.org/10.21638/spbu03.2018.404

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Manuscript Editor: Farida Minibayeva, Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Center, Russian Academy of Sciences, Kazan, Russia

Received: September 28, 2018;

Revised: November 16, 2018;

Accepted: February 4, 2019;

Funding: This work was partially supported by the Russian Foundation of Basic Research (projects No. 18-34-00239).

Competing interests: The authors have declared that no competing interests exist.

expression of genes coding auxin transporters (Ruzicka et al., 2007). Thus, root growth inhibition caused by ethylene and auxins is likely to be either due to ethylene-induced accumulation of auxins in the growing root zones or (alternatively) due to activation of ethylene production by auxins. Recently, preference has been given to the first of these mechanisms. Data on the absence of stimulation of ethylene production by auxin (indoleacetic acid, IAA) in isolated pea roots were obtained about 30 years ago and served as an argument against the participation of ethylene in the root growth response to auxins (Eliasson et al., 1989). Auxin-induced up-regulation of the genes responsible for ethylene synthesis was detected later (Stepanova et al., 2007) but failed to change the opinion on cross-talk between auxins and ethylene. The capacity of ethylene-insensitive *Arabidopsis* mutants to respond to synthetic auxin 1-naphthylacetic acid (NAA- synthetic analog of native auxins) was used as an argument against the importance of the role of ethylene in the root growth response to auxins (Ruzicka et al., 2007). Nevertheless, it is important to emphasize that auxin transporters, which are the likely targets of ethylene action (Negi et al., 2008), are less important for the transfer of NAA across membranes than that of natural auxin indoleacetic acid (Marchant et al., 1999). Thus, analysis of the data on the cross-talk between auxins and ethylene during the control of root growth is still insufficient. We planned to solve this problem by comparing IAA effects on the root growth of ethylene insensitive *Arabidopsis* mutant *etr1-1* and its wild type ecotype Columbia (Col-0). Along with root length, we also measured root fresh weight, since the decrease in root mass of *etr1-1* as compared to Col-0 has been shown by us previously (Korobova et al., 2016), as well as the capacity of root mass accumulation to emphasize plant adaptation (Kudoyarova et al., 2015). We also compared root growth responses to the inhibitor of ethylene perception, 1-methylcyclopropene (1-MCP), in plants with mutation in the auxin receptor TIR1 to that of the wild type genotype. The aim of the present work was to reveal dependence of auxin-induced changes in root growth on their sensitivity to ethylene as well as to discover the action of the inhibitor of ethylene perception under the changes in plant sensitivity to ethylene.

Materials and Methods

Experiments were performed on the plants of *Arabidopsis thaliana* [L.] Heynh. Ecotype Columbia (Col-0), ethylene insensitive mutant *etr1-1* and auxin insensitive mutant *tir1*. After stratification on wet filter in Petri dishes for three days at 4 °C, seeds were transferred to 100-ml pots with sand saturated with the Hoagland-Arnon (H-A) solution and grown in a plant growth cabinet (MLR-350H, “Sanyo”, Japan) at 23°/19 °C (day/night), 80 % air relative humidity, at 120 μmol $м^{-2}$ $с^{-1}$ PAR and 16-h photoperiod, as described by Shtratnikova et al. (2015). Preliminary experiments showed that *etr1-1* grows more slowly at the early stages of development, thus mutants were planted four days earlier than Col-0. Plants received three ml of H-A solution daily plus distilled water according to the pot weight to maintain sand humidity at 60 % of its full capacity, as described earlier (Arkhipova et al., 2007). Two weeks after plant transfer to the growth chamber, we studied the sensitivity of Col-0 and *etr1-1* plants to the addition of exogenous IAA and the sensitivity of Col-0 and *tir1* plants to treatment with 1-MCP. Growing the plants in the sand enabled separation of the roots from the substrate without their damage. To determine sensitivity of growth to exogenous hormone, freshly prepared solution of IAA was added each day to yield a final concentration of 5.7×10^{-6} M, taking into account the volume of nutrient solution in the pot. Daily auxin treatment was necessary due to its rapid breakdown. The concentration was chosen in preliminary experiments as one resulting in the most pronounced response in the plants of wild type. Plants of Col-0 and *tir1* were treated with 1-MCP as described by Vysotskaya et al. (2011). Solution of 1-MCP was prepared from precursor obtained as a gift from its producer (SmartFresh, AgroFresh Inc., USA). The precursor (0.1 g per l) was diluted in 0.05 % solution of the moistening agent Silwett L-77 (De Sangosse Ltd., Great Britain). Leaves of some of the plants were sprayed with the obtained solution in a dose enabling complete moistening of the leaves not later than five min after solution preparation, when gaseous 1-MCP was produced. The second group of plants (control) was treated with the moistening solution. 1-MCP treatment was repeated in four days when new ethylene receptors could form. Immediately after spraying, plants were covered with a polyethylene bag for limitation of gaseous1-MCP diffusion and placed into a dark closed box, since according to Sisler and Serek (1997), irreversible binding of 1-MCP with ethylene receptors takes place in the darkness. In the morning, plants were returned to the growth chamber. Four and seven days after the start of the treatments with auxin and 1-MCP, correspondingly, the mass of all roots of a plant and the length of the longest roots were measured (n = 20). IAA was introduced into the medium daily and could lead to a more rapid manifestation of the growth response. Plant were at the rosette stage when growth characteristics were measured, and the first signs of flowering appeared one week after conclusion of experiments.

Results and Discussion

The roots of *etr1-1* mutants were longer than those of the wild type Col-0 (Fig. 1a). These results can be easily explained by ethylene inhibition of root elongation (Ru-

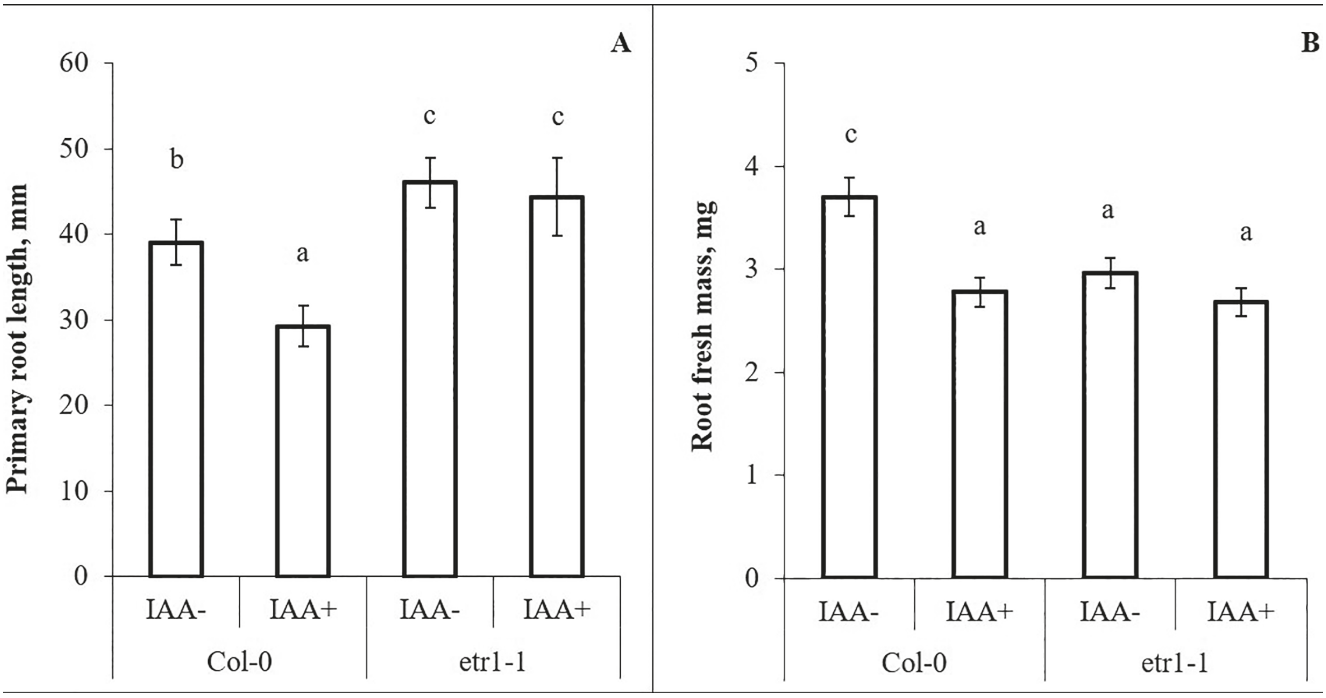

Fig. 1. Primary root length (A) and root fresh mass (B) of 18-days old ethylene insensitive *Arabidopsis* mutant *etr1-1* and its parent ecotype Columbia (Col-0) 4 days after the start of IAA addition (IAA+) to the nutrient solution (to yield final concentration $5{,}7 \cdot 10^{-6}$ M). Statistically different means ($n = 20$) are indicated with different letters (LSD, $p < 0.05$).

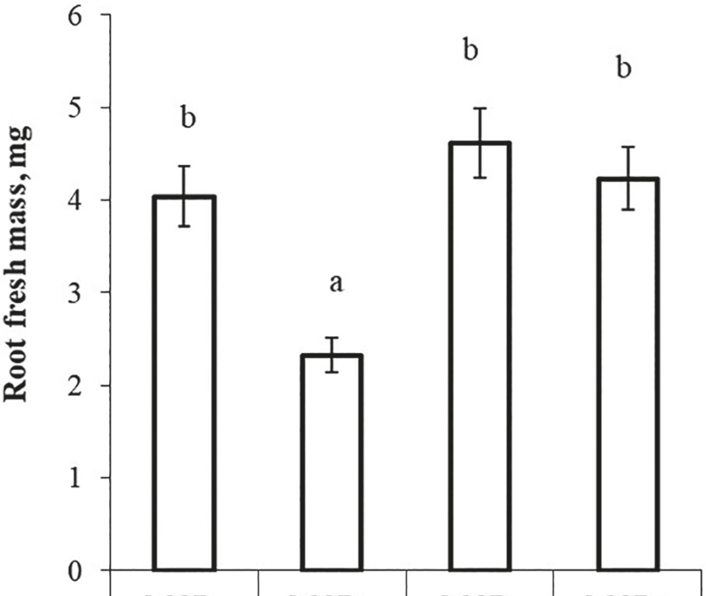

Fig. 2. Root fresh mass of 3-week old auxin insensitive *Arabidopsis* mutant *tir1* and its parent ecotype Columbia (Col-0) 7 days after the start of shoot treatment with inhibitor of ethylene perception, 1-Methylcyclopropene (MCP+). Statistically different means ($n = 20$) are indicated with different letters (LSD, $p < 0.05$).

zicka et al., 2007), while the loss of sensitivity to ethylene accelerates root elongation. Although root length in most publications is presented as a percentage and its absolute values are not provided, longer roots in *etr1* were detected by Contreras-Cornejo et al. (2015). Addition of IAA to the nutrient medium led to 20 % root shortening in Col-0 plants as compared to the control (Fig. 1a). In ethylene insensitive mutants the tendency of 5 % root shortening compared to the control was statistically insignificant. Thus, plant roots that lost their sensitivity to ethylene were unable to respond to IAA in terms of their elongation. Previous experiments showed that the growth response of *etr1-1* plants to synthetic auxin NAA was similar to root growth inhibition in plants of the wild type (Ruzicka et al., 2007). Since IAA crosses cell membranes less easily than NAA and needs transporters to a greater extent (Marchant et al., 1999), the capacity of NAA, but not IAA, to inhibit root elongation of *etr1* mutants confirms the importance of the effect of ethylene on auxin transporters for inhibition of root elongation.

The roots of *etr1-1* mutants were less heavy than those of Col-0 (Fig. 1b). These results are in accordance with the data obtained earlier in similar conditions (Korobova et al., 2016) and in the soil (Tholen et al., 2004). The results indicate that ethylene influences root elongation and mass accumulation in an opposite way. It makes roots shorter, but maintains accumulation of their mass. Thus, root response resembles to some extent the "triple response" detected in the stems and roots of dark-grown germinating seedlings, where ethylene induces their shortening and swelling (Gusman and Ecker, 1990).

Plant treatment with exogenous IAA induced a decrease in root mass of the plants of both tested genotypes. However, the effect was more obvious in the wild type plants: IAA treatment resulted in a 25 and 10 % decrease in the root mass of Col-0 and *etr1-1*, respectively, as compared to the corresponding control ($n = 20$, the difference in the effect of IAA between the two genotypes is significant at $p \leq 0.5$). Thus, the plants with the absence of sensitivity to ethylene retained the capacity to respond to exogenous IAA, manifested in the decrement in root biomass accumulation. Nevertheless, sensitivity to IAA in plants with a mutation of the ethylene receptor was lower than in the wild type plants. These results serve as evidence that IAA by itself (independently of ethylene) may influence root biomass accumulation. However, sensitivity to ethylene increases plants responsiveness to IAA.

Unlike genetic modification of sensitivity to ethylene, inhibition of ethylene perception with the 1-MCP did not significantly change the root length (data not shown). This is likely due to plant spraying with 1-MCP that mostly inactivated leaf, but not root receptors. Treatment of the plants of both genotypes with the inhibitor of ethylene perception (1-MCP) decreased the root mass. Chemical inactivation of ethylene receptors influences root mass accumulation in the same way as switching the receptors off in the mutant. Since, as indicated above, 1-MCP was likely to react mainly with the leaf receptors, the 1-MCP-induced decline in root mass could be a consequence of the effect of ethylene sensitivity on assimilate outflow from the shoots to the roots. Comparison of the growth response to 1-MCP treatment in Col-0 and *tir1* showed that mutation in the auxin receptor decreased the sensitivity of roots to inactivation of ethylene receptors: treatment with 1-MCP resulted in a 40 % decline in the root mass of Col-0 and only a 10 % decline in *tir1* (the difference in the extent of root mass decline between the plants of the genotypes was significant at $p \leq 0{,}01$, $n = 20$).

Conclusion

Our presented results suggest that the capacity of plants to sense ethylene plays an important role in the control of root length and root responsiveness to auxin. Mutations in the gene *ETR1*, which codes the ethylene receptor, contribute to root elongation in *Arabidopsis* and a significant decline in sensitivity of root elongation to exogenous IAA. These results differed from those obtained in experiments with the treatment of the tested mutant with synthetic auxin NAA, which easily diffuses across membranes and (unlike IAA) does need transporters (at least AUX1 (Ruzicka et al., 2007)). The difference between the responses of the ethylene-insensitive mutant to NAA and IAA suggests that the responses to IAA are dependent on ethylene sensitivity due to the effect of ethylene on auxin transporters, which is in accordance with the data of Ruzicka et al. (2007). The loss of sensitivity to ethylene influenced auxin's capacity to inhibit root mass accumulation to a lesser extent than root elongation. Nevertheless, the absence of sensitivity to ethylene in the *etr1-1* mutant decreased responsiveness of root mass accumulation to the inhibiting action of IAA. In a similar way, the limitation of sensitivity to auxins in the *tir1* mutant decreased responsiveness of root biomass to 1-MCP-treatment. These data allow us to suggest an additive action of ethylene and auxins on the accumulation of root biomass and dependence of root elongation regulation by auxins on sensitivity to ethylene.

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